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(54) Title: HEMOREGULATORY COMPOUNDS

(57) Abstract

The present invention relates to novel compounds which have hemoregulatory activities and can be used to stimulate hematopoiesis and for the treatment of viral, fungal and bacterial infectious diseases.

HEMOREGULATORY COMPOUNDS

Field of the Invention

The present invention relates to novel compounds which have hemoregulatory activities and can be used to stimulate hematopoiesis and for the treatment of viral, fungal and bacterial infectious diseases.

Background of the Invention

The hematopoietic system is a life-long cell renewal process whereby a defined stem cell population gives rise to a larger population of mature, differentiated blood cells (Dexter TM. Stem cells in normal growth and disease. Br Med J 1987; 195:1192-1194) of at least nine different cell lineages (erythrocytes, platelets, eosinophils, basophils, neutrophils, monocytes/macrophages, osteoclasts, and lymphocytes) (Metcalf D. The Molecular Control of Blood Cells. 1988; Harvard University Press, Cambridge, MA). Stem cells are also ultimately responsible for regenerating bone marrow following treatment with cytotoxic agents or following bone marrow transplantation.

The major dose-limiting toxicities of most standard anti-neoplastic drugs are related to bone marrow suppression, which if severe and prolonged, can give rise to life-threatening infectious and hemorrhagic complications. Myelosuppression is predictable and has been reported to be dose-limiting in greater than 50% of single-agent Phase I trials cytotoxic compounds (Merrouche Y, Catimel G, Clavel M. Hematopoietic growth factors and chemoprotectants; should we move toward a two-step process for phase I clinical trials in oncology? Ann Oncol 1993; 4:471-474). The risk of infection is directly related to the degree of myelosuppression as measured by the severity and duration of neutropenia (Brody GP, Buckley M, Sathe YS, Freireich EJ. Quantitative relationship between circulating leukocytes and infections with acute leukemia. Ann In Med 1965; 64:328-334).

Synthetic peptides have been reported to induce the synthesis and release of haematoporetic mediators, including m-CSF from bone marrow stromal elements see U.S. Patent Application 08/001,905.

We have now found certain novel non-peptide compounds which have a stimulative effect on myelopoietic cells. They are useful in stimulating myelopoiesis in patients suffering from reduced myelopoietic activity, including bone marrow damage, agranulocytosis and aplastic anemia including patients having depressed bone marrow function due to immunosuppressive treatment to suppress tissue reactions i.e. in bone marrow transplant surgery. They may also be used to promote more rapid regeneration of bone marrow after cytostatic chemotherapy and radiation therapy for neoplastic and viral diseases. They may be of particular value where patients have serious infections due to a lack of immune response following bone marrow failure. They are useful in the treatment and prevention of viral, fungal and bacterial disease.

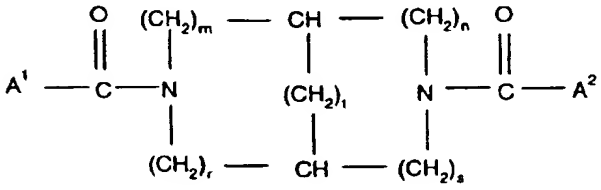
Summary of the Invention

This invention comprises compounds, hereinafter represented as Formula (I), which have hemoregulatory activities and can be used to stimulate hematopoiesis and in the prevention and treatment of bacterial, viral and fungal diseases.

These compounds are useful in the restoration of leukocytes in patients with lowered cell counts resulting from a variety of clinical situations, such as surgical induced myelosuppression, AIDS, ARDS, congenital myelodysplacis, bone marrow and organ transplants; in the protection of patients with leukopenia from infection; in the treatment of severely burned patients and in the amelioration of the myelosuppression observed with some cell-cycle specific antiviral agents and in the treatment of infections in patients who have had bone marrow transplants, especially those with graft versus host disease, in the treatment of tuberculosis and in the treatment of fevers of unknown origin in humans and animals. The compounds are also useful in the treatment and

Detailed Description of the Invention

The compounds of the invention are represented by structural Formula (I)



(I)

A^1 and A^2 are independently $Z\text{-}(CH_2)_k\text{-}(NR^2)_y$-.

Z is independently a 4 - 10 membered mono- or bicyclic heterocyclic ring system containing up to four heteroatoms N, O, S in the ring in which at least one heteroatom is N, and wherein the ring is substituted or unsubstituted by one or two C_{1-4}alkyl, F, Cl, Br, I, C_{1-4} alkoxy, $(CH_2)_mR_4$, oxo, oxime, O-C_{1-4}alkyloxime, hydroxy, $N(R_3)_2$, acylamino or aminoacyl groups, 8, 9, 10 membered monocyclic ring systems being excluded;

R^1 and R^2 is independently hydrogen, C_{1-4}alkylC(O)R_4, C_{1-4}alkyl or R_2 is benzyl which is optionally substituted by one or two C_{1-4}alkyl, C_{1-4}alkoxy, F, Cl, I, Br, OH, or $N(R_3)_2$;

R_3 is independently hydrogen, C_{1-4}alkyl, or benzyl;

R_4 is independently OR_3, $N(R_3)_2$ or SR_3; and

k is an integer from 0 to 4;

m is an integer from 1 to 3;

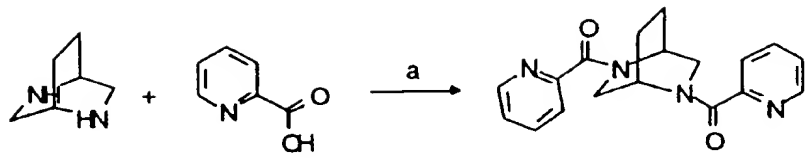
n is 1 or 2;

y is zero or one;

or a pharmaceutically acceptable salt thereof.

C_{1-4} alkyl groups may be straight or branched.

Scheme 1



1 2 3

a) BOP, HOBt, iPr_2NEt, DMF

In order to use a compound of the Formula (I) or a pharmaceutically acceptable salt thereof for the treatment of humans and other mammals it is normally formulated in accordance with standard pharmaceutical practice as a pharmaceutical composition.

According to a still further feature of the present invention there are provided pharmaceutical compositions comprising as active ingredient one or more compounds of Formula (I) as herein before defined or physiologically compatible salts thereof, in association with a pharmaceutical carrier or excipient. The compositions according to the invention may be presented for example, in a form suitable for oral, nasal, parenteral or rectal administration.

As used herein, the term "pharmaceutical" includes veterinary applications of the invention. These compounds may be encapsulated, tableted or prepared in an emulsion or syrup for oral administration. Pharmaceutically acceptable solid or liquid carriers may be added to enhance or stabilize the composition, or to facilitate preparation of the composition. Liquid carriers include syrup, peanut oil, olive oil, glycerin, saline and water. Solid carriers include starch, lactose, calcium sulfate dihydrate, terra alba, magnesium stearate or stearic acid, talc, pectin, acacia, agar or gelatin. The carrier may also include a sustained release material such a glyceryl monostearate or glyceryl distearate, alone or with a wax. The amount of solid carrier varies but, preferably will be between about 20 mg to

Nasal sprays may be formulated similarly in aqueous solution and packed into spray containers either with an aerosol propellant or provided with means for manual compression.

Dosage units containing the compounds of this invention preferably contain .05-50 mg, for example .05-5 mg of the compound of formula (I) or salt thereof.

According to a still further feature of the present invention there is provided a method of stimulation of myelopoiesis which comprises administering an effective amount of a pharmaceutical composition as hereinbefore defined to a subject.

No unacceptable toxicological effects are expected when compounds of the invention are administered in accordance with the present invention.

The biological activity of the compounds of Formula (I) are demonstrated by the following tests.

Induction of Hematopoietic Synergistic Activity in Stromal Cells

The murine bone marrow derived stromal cell line, C6.4 is grown in 12 well plates in RPMI 1640 with 10% FBS. Upon reaching confluence, the C6.4 cells are washed and the media exchanged with fresh RPMI 1640 without FBS. Confluent cell layers of murine C6.4 cells are treated with compound. Cell-free supernatants are collected 18 hours later. Supernatants are fractionated with a Centricon-30 molecular weight cut-off membrane. C6.4 cell hematopoietic synergistic factor (HSF) activity is measured in a murine CFU-C assay.

The following examples are illustrative and are not limiting of the compounds of this invention.

EXAMPLE 1

N,N'-Bis(picolinoyl)-2,5-diazabicyclo[2.2.2]octane

Combine 2,5-diazabicyclo[2.2.2]octane (1 molar equivalent), picolinic acid (3 molar equivalents), BOP reagent (3 molar equivalents), HOBt hydrate (3 molar equivalents) and iPr_2NEt (5 molar eqiuvalents) in DMF. Maintain the reaction at room temperature for 18 h. Quench the reaction by pouring into brine and extracting with EtOAc. Dry the combined organic extracts over Na_2SO_4 and remove the solvent *in vacuo*. Purification by flash chromatography gives the title product.

EXAMPLE 2

N,N'-Bis(pyroglutamoyl)-2,5-diazabicyclo[2.2.2]octane

In the manner of Example 1, using pyroglutamic acid instead of picolinic acid, the title compound is obtained.

EXAMPLE 3

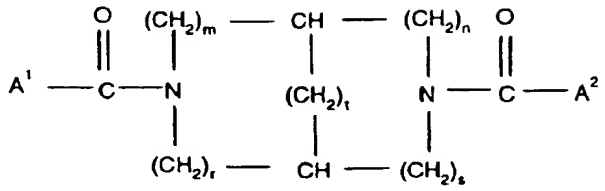
Formulations for pharmaceutical use incorporating compounds of the present invention can be prepared in various forms and with numerous excipients.

Examples of such formulations are given below.

Tablets/Ingredients		Per Tablet
1.	Active ingredient (Cpd of Form.I)	0.5 mg
2.	Corn Starch	20 mg
3.	Alginic acid	20 mg
4.	Sodium alginate	20 mg
5.	Mg stearate	1.3 mg

CLAIMS:

1. A compound of Formula (I)



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R^1 and R^2 is independently hydrogen, C_{1-4}alkylC(O)R_4, C_{1-4}alkyl or R_2 is benzyl which is optionally substituted by one or two C_{1-4}alkyl, C_{1-4}alkoxy, F, Cl, I, Br, OH, or $N(R_3)_2$;

R_3 is independently hydrogen, C_{1-4}alkyl, or benzyl;

R_4 is independently OR_3, $N(R_3)_2$ or SR_3; and

k is an integer from 0 to 4;

m is an integer from 0 to 2;

n is an integer from 1 to 3;

r is an integer from 1 to 3;

s is an integer from 0 to 2;

t is an integer from 0 to 2;

y is zero or one;

or a pharmaceutically acceptable salt thereof;

provided:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/18052

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/435; C07D 401/14
US CL :540/472, 556; 546/113, 121; 514/213, 300
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 540/472, 556; 546/113, 121; 514/213, 300

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

CHEMICAL ABSTRACTS 25-Diazabicyclo [2. 2. 2] octane Vol. 31 - Vol. 124 (1937 - June 1996)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

NONE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4,321,383 (SPRAGUE) 23 March 1982 (23.03.82), see entire document.	1-8

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

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